"A STUDY ON CLINICO PATHOLOGICAL CORRELATION OF HER2 POSITIVITY IN GASTRIC MALIGNANCY"

DISSERTATION SUBMITTED FOR

DM MEDICAL GASTROENTEROLOGY

BRANCH- IV

AUGUST 2014

THE TAMILNADUDR.M.G.R.MEDICAL UNIVERSITY CHENNAI,

TAMILNADU

CERTIFICATE

This is to certify that this dissertation entitled **"A study on clinico pathological correlation of HER2 positivity in gastric malignancy"** submitted by **Dr.Thirumal Perumal** to the Faculty of Medical Gastroenterology, The Tamilnadu Dr.MGR Medical University, Guindy, Chennai-600032, in partial fulfillment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by him under my direct supervision and guidance, during the academic year 2011 to 2014.

Prof. Dr. P. Ganesh, M.D., D.M
Professor and HOD/Guide,
Department of Medical Gastroenterology,
(DDHD@GPH, Annanagar),
Kilpauk Medical College
Chennai

Dr. P. Ramakrishnan, M.D., D.L.O
Dean,
Kilpauk Medical College,
Chennai

DECLARATION

I **Dr.Thirumal P** declare that I carried out this work on **"A study on clinico pathological correlation of HER2 positivity in gastric malignancy"** at the Department of Medical Gastroenterology, Govt. Peripheral Hospital and Kilpauk Medical College. I also declare that this bonafide work or a part of this work was not submitted by me or any other for any award, degree, diploma to any university, board either in India or abroad.

This is submitted to the Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulation for the D.M. Degree examination in Medical Gastroenterology.

Govt. Kilpauk Medical College **Dr.Thirumal P**

Chennai.
24.03.2014

ACKNOWLEDGEMENTS

I am greatly indebted to my guide **Dr. P .Ganesh, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me the chance to undertake this dissertation work under his guidance. Also I express my deep sense of gratitude for his constant encouragement, directions, periodical discussions, rigorous reviews and precious suggestions that helped in the shaping of my dissertation.

I express my sincere gratitude to **Dr. S. Jeevan Kumar, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me permission to do this dissertation in Govt. Peripheral Hospital, Anna Nagar, Chennai-102 and also his kind encouragement and review of my work, besides providing me with all the required facilities.

I also thank **Dr. T.Rajkumar Solomon, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical college, Chennai for his constant encouragement and suggestions for my study.

I am extremely grateful to **Dr.P.Ramakrishnan, M.D., D.L.O,** Dean, Kilpauk Medical College for granting me permission to do this dissertation in Kilpauk Medical College, Chennai.

I am very much thankful to **Dr.M.Saraswathi,M.D.,** Professor and HOD, Department of Pathology, Kilpauk Medical College, Chennai and pathologist of Medall diagnostic centre, chennai who have helped me a lot during my dissertation..

I am also extremely thankful to **Dr.R. Balamurali, M.D., D.M., Dr.G.Ramkumar, M.D., D.M.,** and **Dr.K.Muthukumaran, M.D., D.M.,** who guided me a lot.

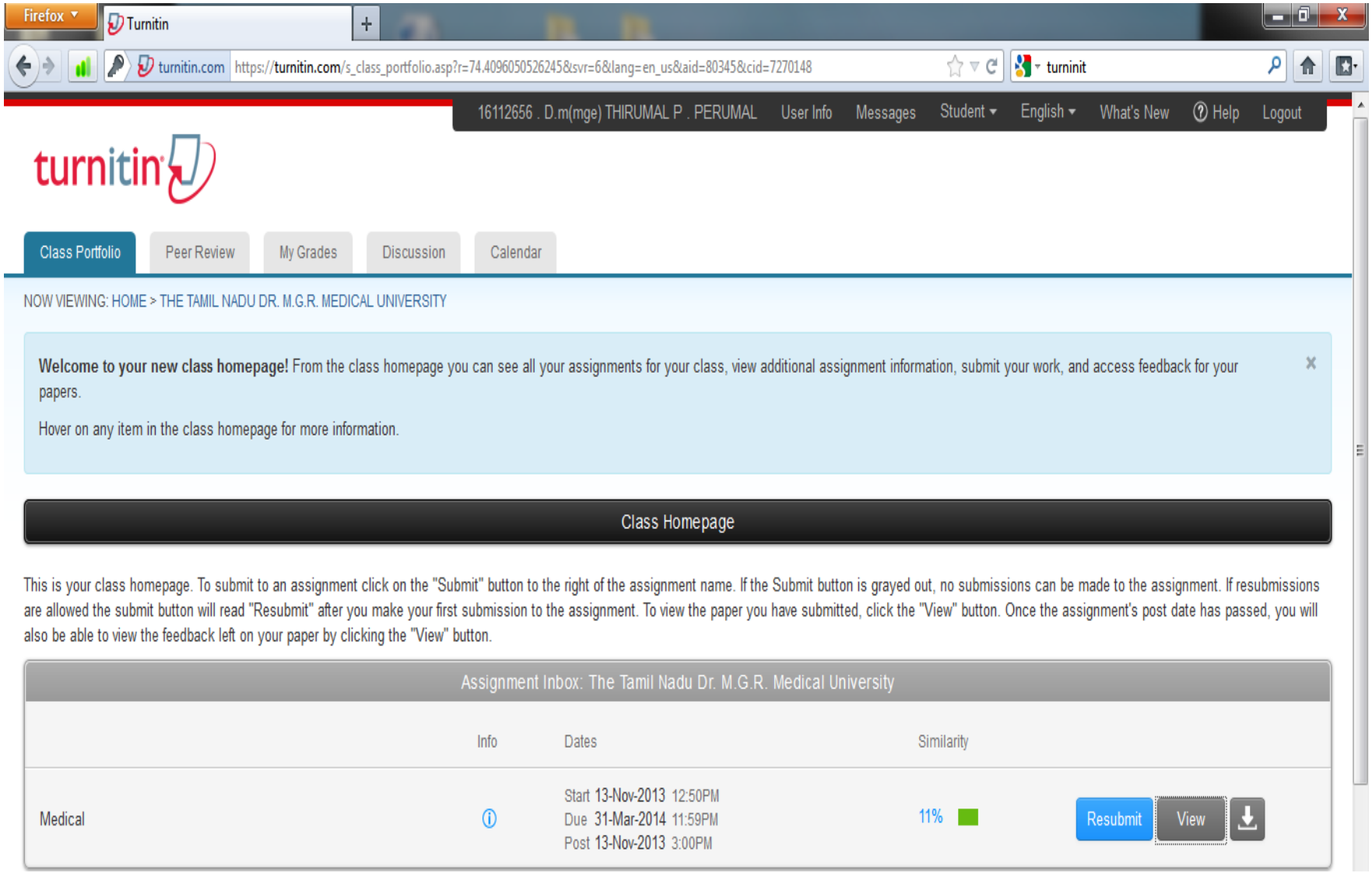
I am also very thankful to all my **Fellow Residents** who have helped me in this dissertation.

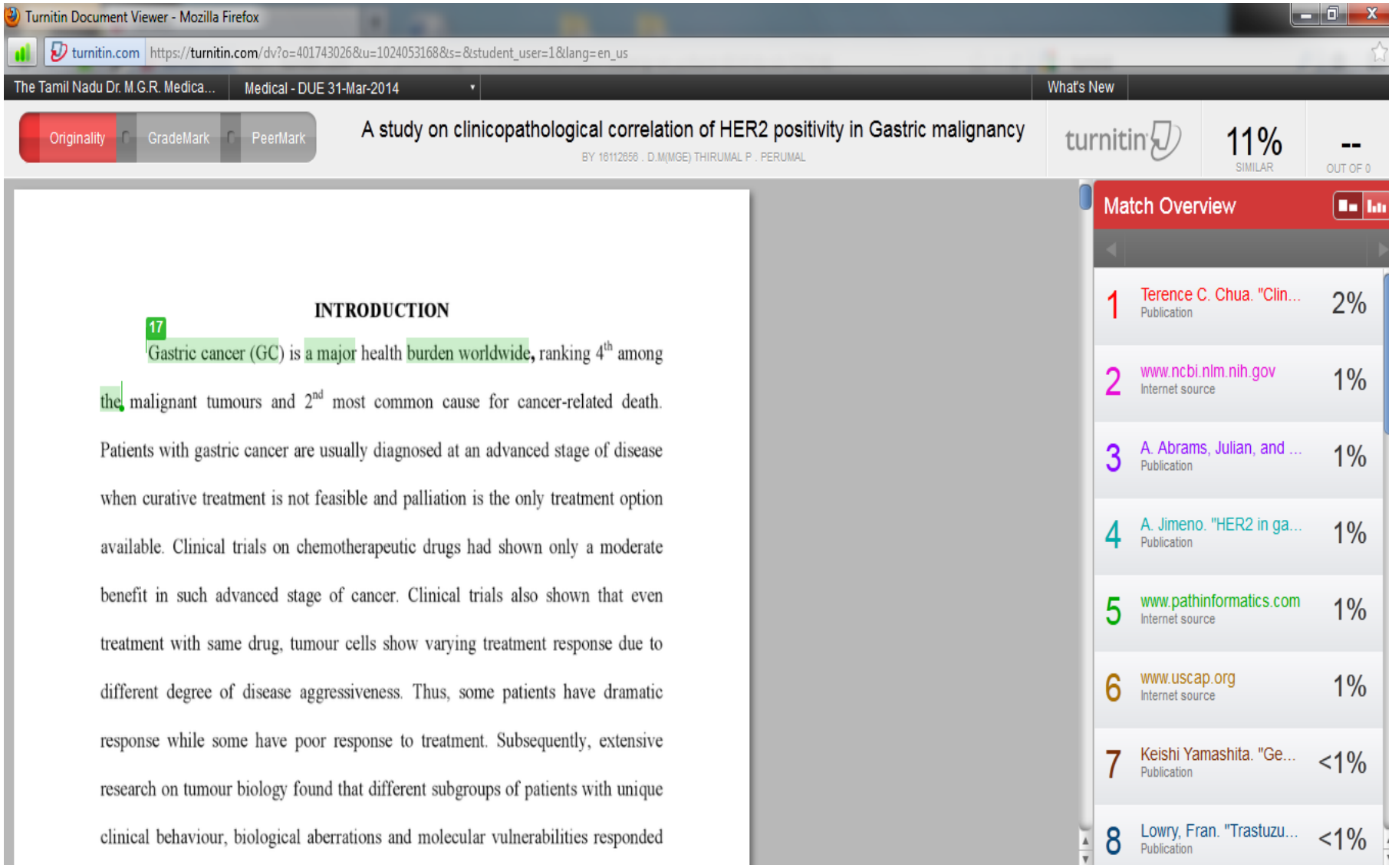
I am also thankful to **Dr.Balaji,** Assistant Professor, Department of Social and Preventive Medicine, SRM College, Chennai for his help in the statistical analysis of my dissertation work.

I thank all the patients who voluntarily participated in this study, without whom this study would not have seen the light of the day.

I also thank all the paramedical staff attached to Govt. Peripheral Hospital, Anna Nagar, Chennai who have helped me in doing this dissertation work.

Finally, I would like to thank my wife, **Dr. PoomathyThirumal**without whose cooperation this work would not have been possible.


Firefox
Turnitin
turnitin.com
https://turnitin.com/s_class_portfolio.asp?r=74.4096050526245&svr=6&lang=en_us&aid=80345&cid=7270148
turninit
16112656 . D.m(mge) THIRUMAL P . PERUMAL
User Info
Messages
Student
English
What's New
Help
Logout
turnitin
Class Portfolio
Peer Review
My Grades
Discussion
Calendar
NOW VIEWING: HOME > THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.
Class Homepage
This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your first submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.
Assignment Inbox: The Tamil Nadu Dr. M.G.R. Medical University
Info
Dates
Similarity
Medical
Start 13-Nov-2013 12:50PM
Due 31-Mar-2014 11:59PM
Post 13-Nov-2013 3:00PM
11%
Resubmit
View


Turnitin Document Viewer - Mozilla Firefox
turnitin.com https://turnitin.com/dv?o=401743026&u=1024053168&s=&student_user=1&lang=en_us
The Tamil Nadu Dr. M.G.R. Medica...
Medical - DUE 31-Mar-2014
What's New
Originality
GradeMark
PeerMark
A study on clinicopathological correlation of HER2 positivity in Gastric malignancy
BY 16112656 . D.M(MGE) THIRUMAL P . PERUMAL
turnitin
11%
SIMILAR
--
OUT OF 0
INTRODUCTION
17
Gastric cancer (GC) is a major health burden worldwide, ranking 4th among the malignant tumours and 2nd most common cause for cancer-related death. Patients with gastric cancer are usually diagnosed at an advanced stage of disease when curative treatment is not feasible and palliation is the only treatment option available. Clinical trials on chemotherapeutic drugs had shown only a moderate benefit in such advanced stage of cancer. Clinical trials also shown that even treatment with same drug, tumour cells show varying treatment response due to different degree of disease aggressiveness. Thus, some patients have dramatic response while some have poor response to treatment. Subsequently, extensive research on tumour biology found that different subgroups of patients with unique clinical behaviour, biological aberrations and molecular vulnerabilities responded
Match Overview
1 Terence C. Chua. "Clin... Publication 2%
2 www.ncbi.nlm.nih.gov Internet source 1%
3 A. Abrams, Julian, and ... Publication 1%
4 A. Jimeno. "HER2 in ga... Publication 1%
5 www.pathinformatics.com Internet source 1%
6 www.uscap.org Internet source 1%
7 Keishi Yamashita. "Ge... Publication <1%
8 Lowry, Fran. "Trastuzu... Publication <1%

CONTENTS

INTRODUCTION

Gastric cancer(GC) is a major health burdenworldwide**,** ranking 4th among the malignant tumours and2nd most common cause for cancer-related death. Patients with gastric cancer are usually diagnosed at an advanced stage of disease when curative treatment is not feasible and palliation is the only treatment option available.Clinical trials on chemotherapeutic drugs had shown only a moderate benefit in such advanced stage of cancer. Clinical trials also shown that even treatment with same drug, tumour cells show varying treatment response due to different degree of disease aggressiveness.Thus,some patients have dramatic response while some have poor responseto treatment. Subsequently, extensive research on tumour biology found that different subgroups of patients with unique clinical behaviour,biological aberrations and molecular vulnerabilities responded well to specific targeted chemotherapeutic agents.

One such subgroup,tumor cells with overexpression of HER2protein were found to respond to targeted molecular therapy against HER2. Monoclonal antibody against HER2 receptor protein which modifies the signaling cascades involved in cell differentiation, proliferation and survival have shown better survival benefit in this subgroup of patients.One such anti HER2 agent is Traztuzumab and this agent had shown significant survival benefit in patients

with HER2positive breast cancer. This agent isalso being tried in the subgroup of gastriccancer patients and shown survival benefits.

Chua et al in a systemic review of 49 studies involving 11,337 patients reported HER2overexpression inup to 53%, with a median of 18% in patients with gastric cancer. This overexpression was found to be associated with poorer survival.

Lancet 2010; 376: 687–97 published that chemotherapy incombination withTrastuzumab can be considered as a new standard option for patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer and appropriate patient selection by HER2 IHC testing will now be part of routine pathology.

Hence, we attempt to study the incidence,clinical, pathological characteristics of HER2 positivity in gastric malignancy in our population and to interpretits role in gastric malignancy.

REVIEW OF LITERATURE

Gastric cancer(GC) is a major health burden worldwide,ranking 4th among the malignant tumors and 2nd most common cause for cancer-related death(10%).GC accounts for 8% of all newly-diagnosed cancer. There is wide variation in incidence of GC worldwide with higher incidence rates reported in East Asia(40 -60 per 100,000) and lower rates in Africa and north America (0.3 -3 per 100,000).In recent decades,it has been observed that there is gradual decrease in incidence in many population.However,not all types of gastric cancer are declining; tumor of cardia and esophagogastric junction are in increasing trend.Recently,an unexplained increase in younger individuals (<40 yrs) has been reported.

GLOBOCAN 2008 is the latest available source that gives figures of GC worldwise. About 73% of gastric cancer cases occur in Asia and almost half (47%) of world's total occurs in china.The incidence rate varies worldwide being 60 per 100,000 in japan and korea to less than 6 per 100,000 in north America. High rates may also be due to good surveillance and detection of very small lesions.Based on this registry the age standardized incidence rate in india are 11.9 and 5.8 per 100,000 among males and females respectively.Male to female ratio in incidence rates is 2: 1 world wide consistently.

The reason for regionalvariation of incidenceof gastric cancer is multifactorial and wouldnot be attributed to a single factor like ethnicity. Environmental and dietary factors are found to have a strong influence on the varied geographical distribution. This strong influence is best demonstrated by epidemiological studies,in that, immigrants from high prevalence areas to low prevalence areas were observed to have reduced risk for gastric cancer.

In India, based on the data from the National Cancer Registries, gastric cancer is a leading problem in North-eastern and Southern states of Indian. A nationally representative survey done recently revealed that second most common fatal cancer with mortality of 12.6% among 556,400 deaths in 2010.[1]

North-eastern registries (Mizoram registry) mentioned the highest rate of gastric cancer in India being 50.6 per 100,000 than the rest of the country. Overallthe gastric cancer incidence in India is less in comparison to other regions of the world. The age-adjusted rate (AAR) of gastric cancer among urban registries in India is (3.0–13.2) compared to the worldwide AAR (4.1–95.5).[3] The age-adjusted incidence rate of stomach cancer in males varies widely among registries, highest being 11.1 per 100,000 in Chennai compared to 1.6 per 100,000 in Bhopal.[5] Similar to trends of stomach cancer globally, Indian registries have also observed statistically significant decreasing trend over the last 20-year.The difference in age of presentation(a decade earlier in south India) can be attributed to the regional difference in incidence and presentation.[6]Among the predisposing factors studied, varied dietary pattern

along with alcohol and tobacco were considered potential risk factors .A study from Chennai found that one of the independent risk factors for gastric cancer were alcohol consumption and use of pickled food.[4] On the other hand, use of pulses was found to be offering protection.

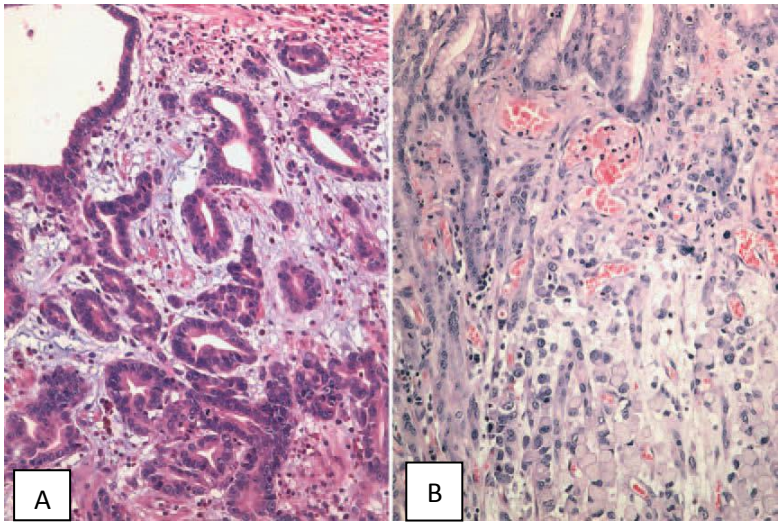
Distal gastric cancers were detected more commonly in individuals from lower socio-economic groups and developing countries like India. In patients with distal tumors,Helicobacterpylori (H. pylori) infection and varied dietary pattern were the mostcommon risk factors.Gastro esophageal reflux disease and obesity are said to be major risk factors for the development of proximal cancer which predominates in developed countries and higher socioeconomic groups. But, studies from areas of high incidence of H. pylori infection like Asia and Africa had shown there is no linear correlation between rate of H. pylori infectionand incidence of gastric cancer. This Asian or African paradox suggests that H. pylori by itself cannot cause gastric cancer and various other factors are needed for causation.[7]Thus, various etiological factors including smoking, alcohol, nitrates, and *H. pylori* have been proposed as causative factors for gastric cancer.

From these epidemiological studies, it implicates that gastric cancer is not a single disease or caused by a single factor, but a combination of genetic, sociocultural, and environmental factors in a given region and that dictates its presentation.

PATHOLOGY

Any malignant tumor that arise in stomach that involve regionfrom the gastro-esophageal junction to the pylorus is referred as Gastric cancer(GC). Tumor of epithelial origin,adenocarcinoma constitutes about 95 per cent of stomach tumors. Other epithelial tumors like adenosquamous, squamous and undifferentiated carcinomas are rare.[8]Gastric cancer was broadly classified in mid 1960s as intestinal or diffuse type as proposed by Lauren *et al*. based on histological findings.Each type has distinct clinical and epidemiological pattern of presentation.The well-differentiated intestinal-type have neoplastic cells that is cohesive and forms tubular structures resembling gland. This type of tumor usually ulcerates. On the other end,poorly differentiated diffuse-type is characterized by thickened stomach wall due to infiltrationof tumor diffusely without discrete mass and thisresembles like a leather bottle in barium study. Epidemiologically, intestinal-type, is more common in men of old age with high prevalence in African-American with betterprognosis. And, this type of tumor generally arises from precancerous lesions like atrophic gastritis and intestinal metaplasia and influenced by environmental factors like H. pyloriinfection, high salt intake,obesity.The diffuse-type is the more prevalent in endemic areas and present in younger age with genetic susceptibility. Some tumor cells have both intestinal and diffuse type of cells and are termed as mixed gastric carcinoma.

Histopathologic types of gastric cancer.
A, The intestinal type of gastric adenocarcinoma is characterized by the formation of tubular structuresmimicking intestinal glands.
B, The diffuse type of gastric adenocarcinoma contains singly invasive tumor cells that



Clinicopathologic features of intestinal and diffuse types of gastric adenocarcinoma.

	Intestinal Type	**Diffuse Type**
Gender	Males >females(2:1)	Males = females
Age	Older age(> 50yrs)	Younger age(< 50yrs)
Areas	High risk(Japan,china)	Uniform across the world.
Incidence	Decreasing	Increasing
Inheritance	Hereditary nonpolyposis colorectal cancer,FAP	Hereditary Diffuse gastric Cancer
Location	Antrum(Distal)	Corpus/body,anywhere
Growth Pattern	Usually exophytic	Flat,ulcerated,linitis plastic
Histology	Gland formation,extracellularmucin.	Loss of

		cohesion,signet ring cells,no. glands.
Precursors	Correa pathway:Chronic H.pylorigastritis,atrophy,metaplasia ,dysplasia.	Carneiro pathway: hereditary diffuse gastric cancer.

In 2010, WHO revised the classification according to the morphological patterns commonly exhibited by the tumor into five major types 1.Papillary 2.tubular 3.mucinous (mucinous poolexceeding 50% percent of tumor) 4.poorly cohesive (signet ring cell typeand other variants) and 5.mixed adenocarcinoma.[9]

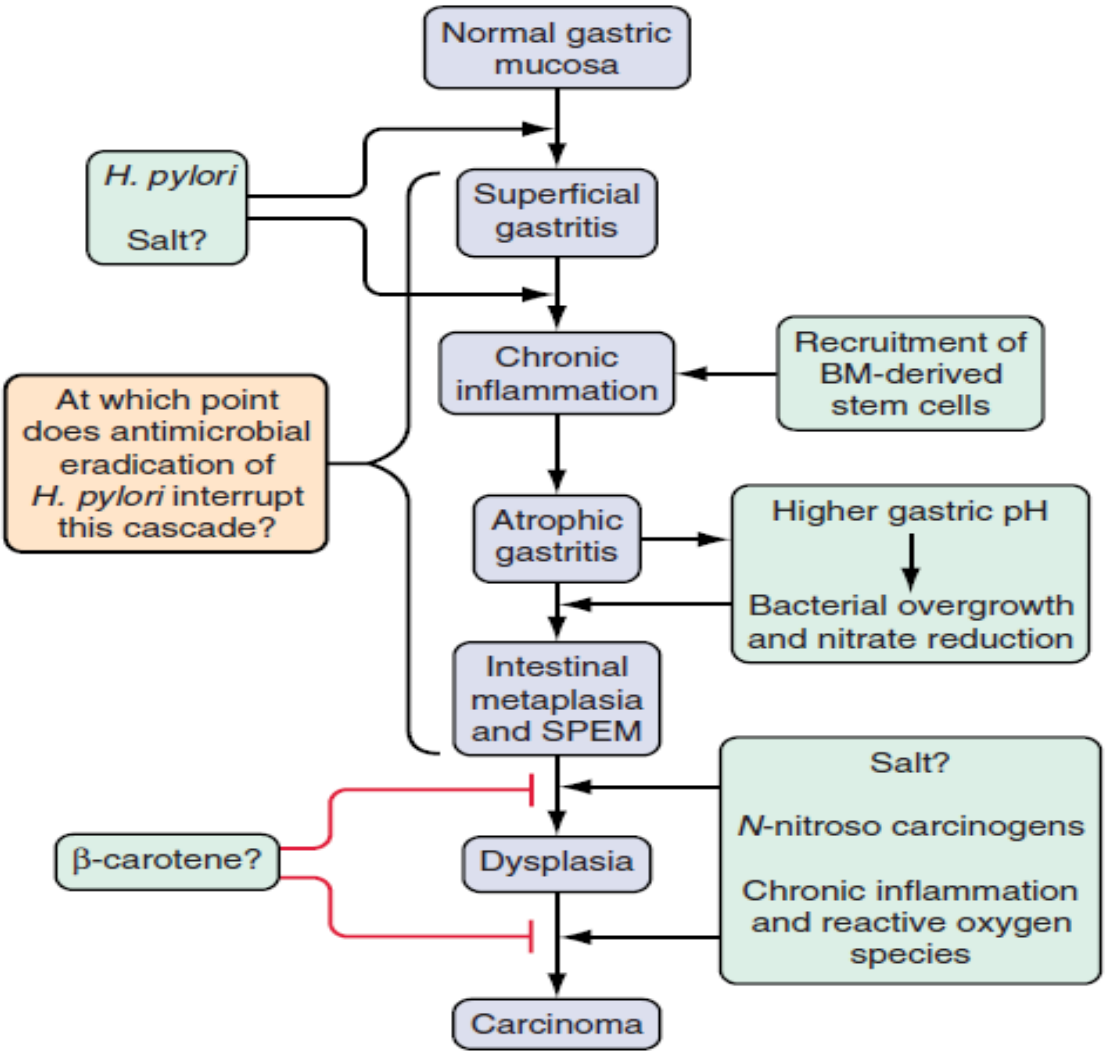
Lauren and WHO classification of gastric cancer

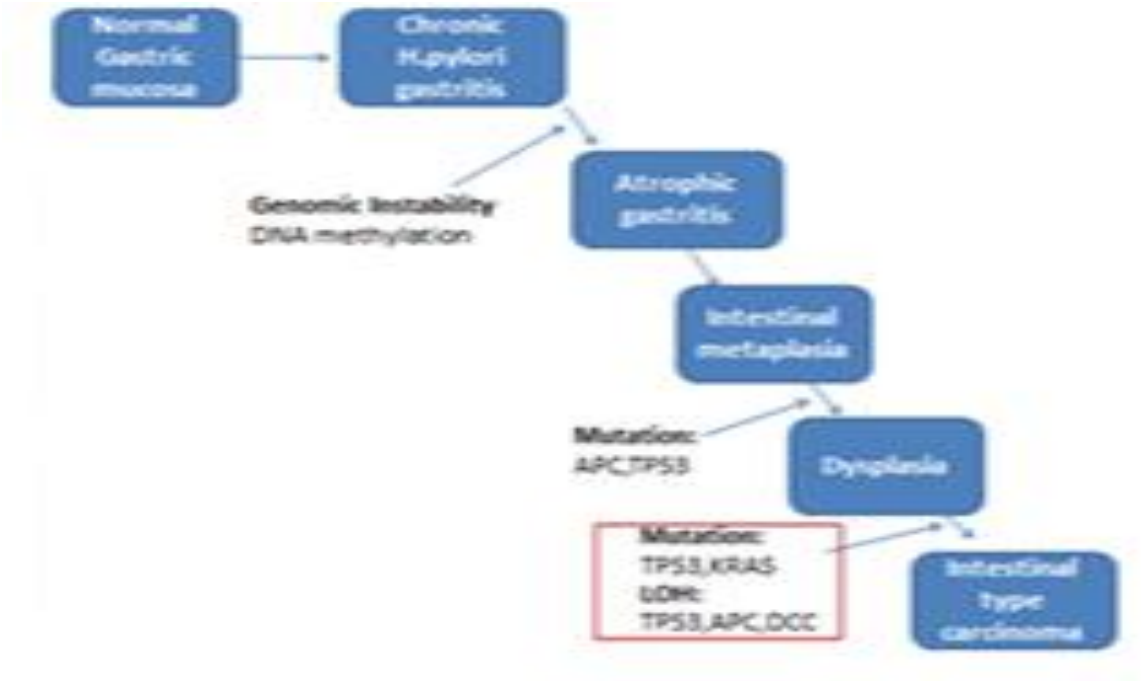
Lauren	**WHO classification 2010**
Intestinal Type	Papillary adenocarcinoma Tubular adenocarcinoma Mucinous adenocarcinoma
Diffuse Type	Poorly cohesive carcinoma(Signet ring and its variants)
Mixed(equal intestinal and diffuse)	Mixed type(glandular and poorly cohesive)
Indeterminate	Undifferentiated carcinoma
	Adenosquamous carcinoma Carcinoma with lymphoid stroma Hepatoid adenocarcinoma Squamous cell carcinoma.

NCCN 2013 guidelines for management of gastric cancer uses American joint committee on cancer staging system which grade gastric cancer in 4 grades as follows grade G1 = well differentiated ,G2 = Moderately differentiated G3 = Poorly differentiated and G4 = Undifferentiated based on histological pattern.

Invasive gastric carcinoma develops from precancerous lesions through stepwise evolution that include atrophic gastritis → intestinal metaplasia → dysplasia → carcinoma.Intestinal type gastric cancer follows this metaplasia/ dysplasia/carcinoma sequence that develops as a result of series of alterations/mutation in gene.

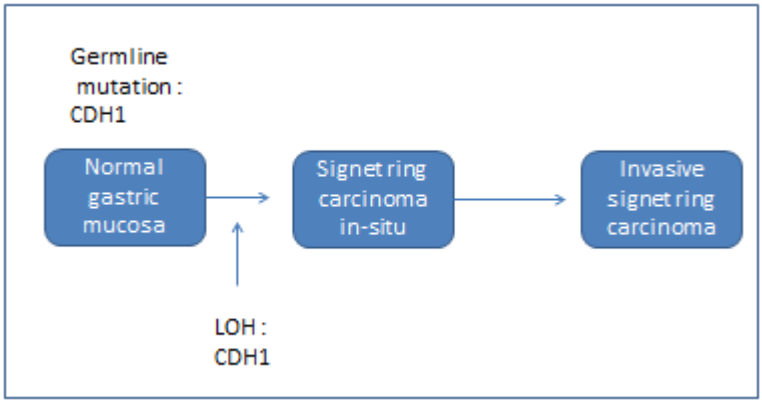
Fig.A Correa cascade of gastric carcinogenesis.





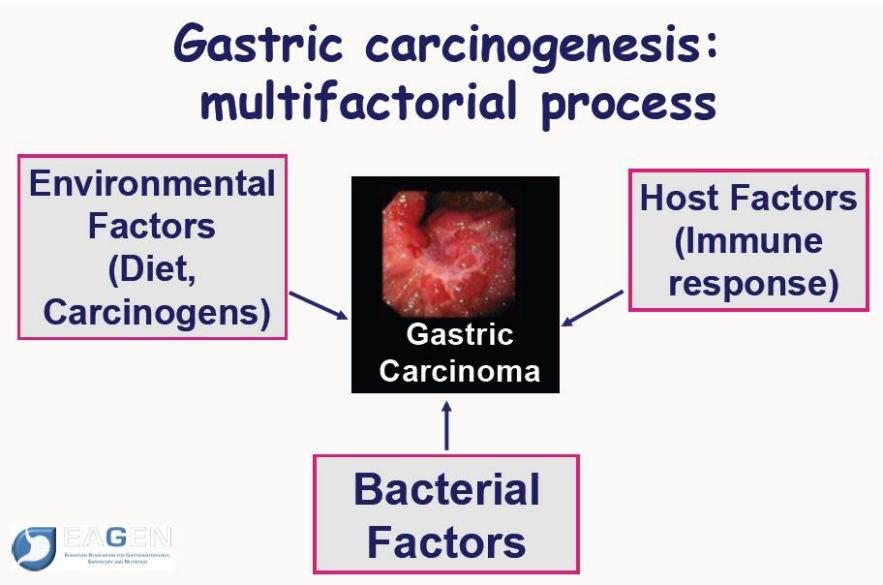
Diffuse type of gastric cancer are mostly sporadic however ,familial clustering is present in 10% and 1% to 3% of gastric cancers arise as a hereditary syndrome.These tumors follow different pathway of cancer genesis described as carneiro model as follows

Fig .B The Carneiro modelof hereditarydiffuse gastric cancer.



Etiology

Gastric cancer is a disease of complex etiology involving multiple risk factors and multiple genetic and epigenetic alterations.



Even though many factors are postulated as etiology for development of gastric cancer, H.pyloriinfection have been attributed to about 80% of cases. Other factors such as dietary habits, lifestyle habits, genetic factors and socioeconomic status also have contribution to cancer formation.

Helicobacter pylori.

H. pylori isa spiral,microaerophilic,gram negative bacteria that resides in gastric mucosa of patients and cause gastritis leading to chronic atrophic gastritis. There is two times increased risk of gastric cancer development in patients with H.pylori infection as evidenced by various meta-analysis.[10]

The mechanisms by which H. pyloricontributes to carcinogenesis has been extensively studied, andit has been found that virulent strains in a permissive microenvironment induced cancer in a genetically susceptible host.

The sequential progression of changesfrom normal gastric epithelium to carcinoma as described above had been suggested to be triggered by H.pylori.[11-13]The products like urease,phospholipase,ammonia secreted by the bacteria cause mucosa damage and loss of barrier function through activation of urease-mediated myosin II.[14]Oxidative stress generated by this products acts as virulence factor and leads to production of oxygen free radicals and nitrogen species. This redox potential cause oxidative DNA damage. The damage due to oxidative stress occurs only in susceptible host with H.pylori infection.[15]

H. pylori does not cause direct mutation in host cell but favors the production of such substances through cytokines release. Another mechanism speculated for mutagenicity isimpairment of mismatch repair pathway.[11,16]These mutations in nucleus and mitochondria leads to aberrant DNA methylation. This epigenetic alteration (aberrant DNA methylation) is important inducer of carcinogenesis in gastric cancer.

The cag pathogenicity island (cagPAI)containing strains of H.pylorihas been found to be most virulent by various epidemiological studies.Other virulence factors demonstrated are SabA,BabA,VacA.The CagA proteinbecomesphosphorylatedafter binding to gastric mucosa through tyrosine phosphorylation by SRC family kinases. This Phosphorylated CagAtransmits positive signals for cell growth and motility by selectively binding and activating SHP2, a phosphatase.This further activates translocation of B-catenin

and p120 to nucleus,altering transcription of genes leading to increased proliferation, angiogenesis and inhibition of apoptosis.[Figure.C].

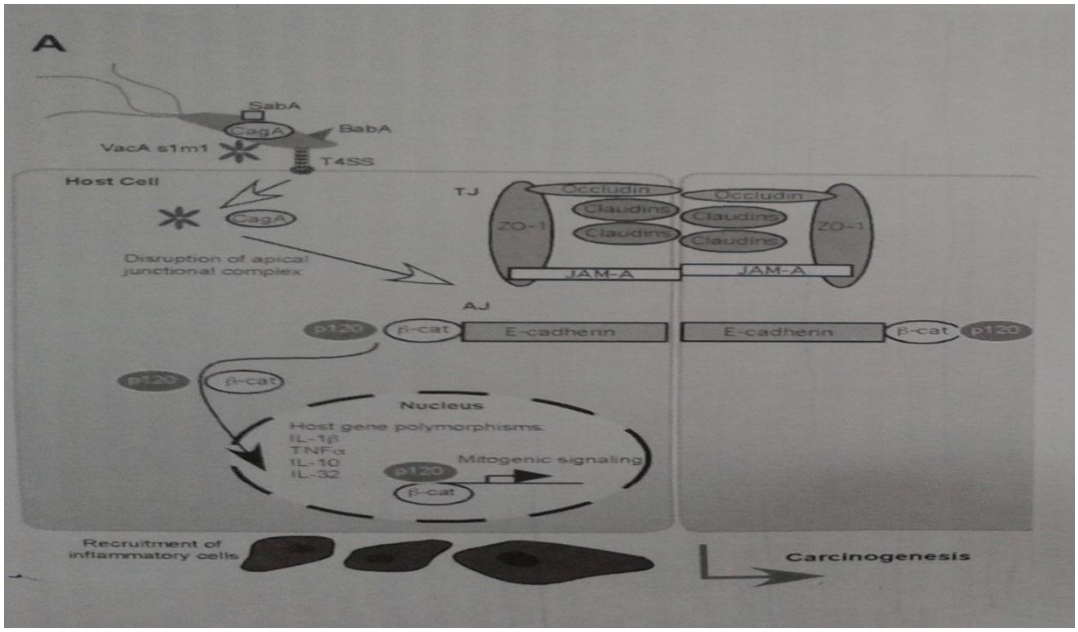


Fig :CH.pylori virulence factors SabA,CagA,BabA and VacAinflunces outcome of infection.

CagA and VacAs1m1 types increases the disease severity.*cagA*activate translocation of B-catenin and p120 to nucleus,altering transcription of genes that promote disease progression

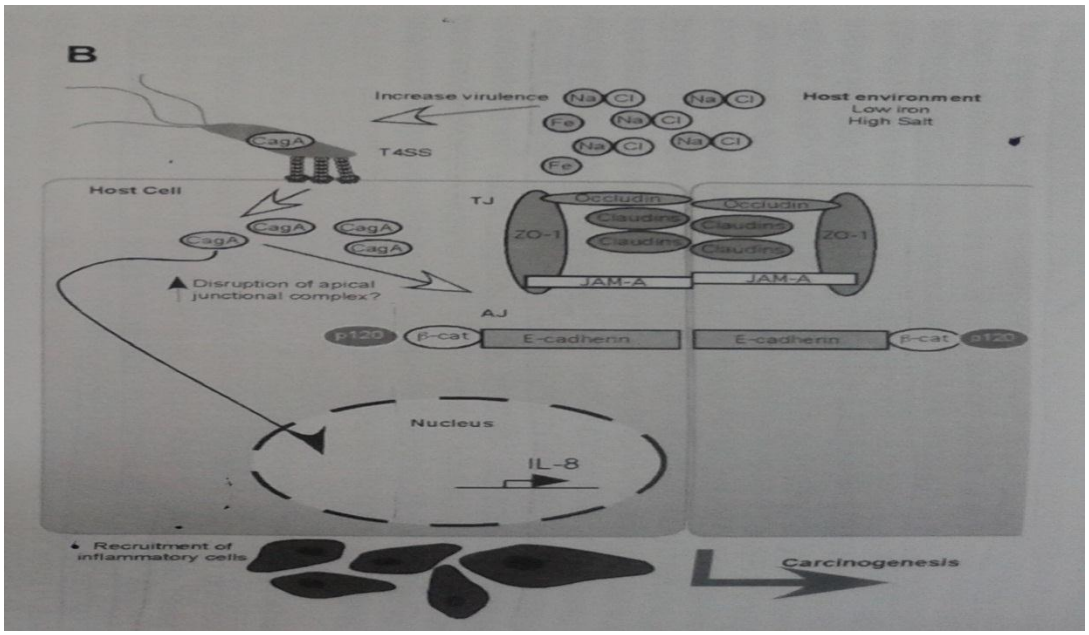
Host genetic diversity also contributes to cancer,including polymorphisms within IL-1b,IL- 10 and IL - 32

Dietary factors

Excessive salt intake plays a strong role in gastric carcinogenesis as evidenced by many epidemiological and experimental studies. Increased consumption of dietary salt is reported to increase the risk of cancer development evidenced by themeta-analysis of prospective studies done by D Eliaet al.[18]Salt cause direct damage of gastric mucosa and results in gastritis

which increases risk. Anotherhypothesis is that salt induceshypergastrinemia and this leads parietal cell proliferation and tumor progression.

Dietary nitrates are also found to contribute to carcinogenesis. Mutagenic compounds are produced guanidine, L-arginine-containing polypeptides thatfound in natural food undergo nitrosation and if nitrate in food come in contact to gastric acid.Other dietary factor found to have association is the one found in smoked food -benzo[a]pyrene.[9]



Host iron(Fe) levels and salt (NaCl)concentrations also affect virulence of H.Pylori.

High salt increases cagA production and low iron levels increase assembly of T4SS pili,cagA translocation and IL-8 secretion.

Lifestyle

Sjödahlet al[20] in his population-based prospective cohort study observed that intake of alcohol and tobacco directly increase the risk of

development of gastric cancer.The mechanism by which smoking perpetuate the carcinogenesis is by decreasing prostaglandins that maintain integrity of gastric mucosa.

H.pylori incidence is higher in smokers than non-smokers. A European study[21] reported that there is significant association between smoking duration and intensity with gastric cancer development.The mechanism for injury induced by smoking was decreased production of prostaglandins that maintains mucosal integrity. A recent study had shown that in patients who had curative surgical resection for cancer, continuous smoking habit was found to be a significant independent risk factor for death from gastric cancer [22.]

GENETIC ANDEPIGENETIC ALTERATIONS.

The study on gastric carcinogenesis is an evolving field in research. Mechanism of cancer development is a complex biology and involves multiple step whereby normal cell is transformed to tumor cell with invasiveness. Alterations in various genes that control cell growth like tumor suppressor genes, DNA repair genes, cell cycle regulators, and signaling molecules has been demonstrated in gastric cancer.

Recent studies had shown that fordevelopment of gastric canceran average of 4.18 genomic alterations are necessary.[23] Genomic alterations and subsequent Genomic instability may be due to either chromosomal instability(CIN)or microsatellite instability (MSI).

Chromosomal Instability(CIN)

Chromosomal aneuploidyis the common chromosomal abnormality that most gastric cancer exhibits. Sasaki et al reported that aneuploidy was present in both differentiated tumors&undifferentiated tumors in about 72% and 43% respectively.

Comparative genomic hybridization(CGH) analyses have revealed several changes in copy number of DNA. Decreased copy number was exhibited in chromosomal arms 4q, 5q, 17p&18p and increased number of copy in chromosomes 8q, 17q& 20q.Analyses also shown that 20q gains and 18q losses were more frequently seen in tumor that metastases to lymph node.

Analysis by comprehensive loss of heterozygesis(LOH) have revealed that 3p,4p,8q,17p& 18q were lost in majority of tumor cells. Younger patients have shown to have numerous variations in DNA number of copy and gains inthese chromosomal regions 6p21, 9p34, 11q23, 17p13, 19p13 & 22q13.[25]

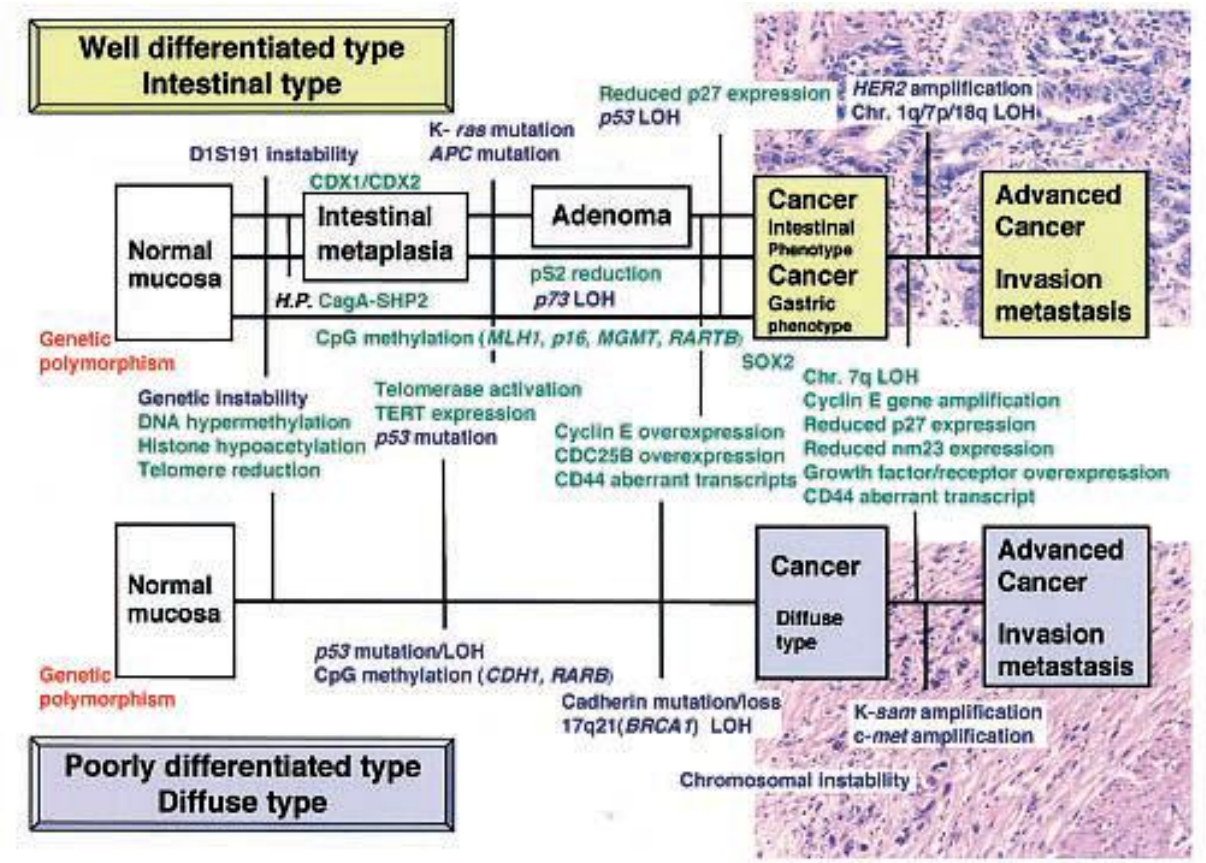


Fig:DMultiple genetic and epigenetic alterations during gastric carcinogenesis

Gastric cancers with familial clusteringshows microsatellite instability(MSI),that results from errors in DNA replicationin about 15 – 20 percent.Intestinal-type gastric cancer of advanced stage with invasiveness was associated with epigenetic inactivation of the gene hMLH1[26]whereas sporadic cancer with less invasiveness was associated with mutations in transforming growth factor-β (TGF-β) RII,BAX genes and insulin-like growth factor-II(IGF-II) R.

Acquired somatic genetic/Molecular alterations.

Oncogenes

Oncogenes alterations due to mutation like activation /overexpression has been has been documented in gastric cancer.Intestinal- type cancer and its precursor lesions were demonstrated to have mutated K-ras oncogene in codon-12. Anotherdemonstrated alteration is overexpression of a cell surface receptor –

c-erbB2/HER2in intestinal-type gastric cancer.[28]In diffuse-type gastric cancers, aberrations in the FGFR2/ErbB3/PI3 kinase pathway andamplification of c-met have been frequently documented.[28]

Tumor Suppressor genes.

TFF -1 loss

More than 50% of gastric cancers has been demonstrated to have 'loss of Trefoil peptide TFF1(pS2)'.Gastrokine(GKN) 2,a stomach specific tumor suppressor protein was structurally and physically similar to TFF1 and loss of GKN1 and GKN2 expression was found in gastric adenocarcinoma.

p53 gene

Altered p53 gene is consistently seen in gastric adenocarcinoma with loss of allele in 60% of cases and about 30 to 50% of cases shown mutation.[30]

Cell cycle regulators, growth factors and cytokines

Gastric tumor cell produce many growth factors and cytokines which controls the cell growth, differentiation and activation throughTGF-B pathway. Dysplastic cells shown increase in the expression of Transformation growth factor (TGF) beta -1/2, TGF-beta R1, MYC &TP53 along with overexpression of SMAD4, CDKN1A, SMAD2/3 &CDKN1B while overexpression ofTGF – beta R2, SMAD7, RELA&CDC25A had been observed in both dysplasiaand carcinoma.[31]Many meta -analysis and reviews reported that risk of gastric cancer increases with presence of gene polymorphisms atpositions -511, 31 and +3954 in Interleukin (IL)-1beta cluster.[32]

Others

E-cadherin alteration (reduced expression), Methylation silencing alterations(CpGhyper methylation) and apoptosis signaling alterations (mutation of BAX gene) were demonstrated inabout 26%,41% and 33% of gastric adenocarcinoma respectively.[33,34]

Biomarkers : For Diagnostic,Prognostic,Treatment Guidance.

The high mortality rate in GC is due to delayed detection and surgical resection at advanced stages of disease.Currently,detection of early tumors is limited as there are no reliable markers.Major research is being made to develop

the molecular signature based methods complementing histo pathological diagnosis that can prognosticate the patient.

Various biomarkers like CA 10-9,CEA,phospholipase A2,TGf-B1,VEGF and others had been tried but all shown to have poor sensitivity and specificity.

Serum markers like tissue inhibitor of metalloproteinase(TIMP-1),interleukin-10,hepatocyte growth factor, interleukin-2 soluble receptor and E-cadherin soluble fragment have been reported in more invasiveness tumors with poor survival.[35,36]

Serum Proteomic evaluation of GC patients revealed that a panel of 5 proteinmarker consisting of Ago2 miRNA,MiR-187,miR-371-5p,miR-378 and miR-486 shown to have sensitivity and specificity of 83% and 95% respectively.[37].But,this need validation since this study had small sample sizeand were mostly retrospective and case control study.

Chemosensitization Markers

Response to specific treatment agents can be predicted from identification of molecular markers in tumor cells. Markers like overexpression of p53,BAX and Bci-2 staining was observed in small group of patientsand that showed poor response and chemoresistant to currently available chemotherapy agents.(38,39).

With good molecular analysis and better understanding of cancerogenesis,molecular targeted therapies are being developed.One such

molecular target identified was HER2/neu receptor, against which therapeutic agent showed improved survival.

The protein,HER2/neu (ERbB-2) is a 185-kDa trans-membrane tyrosine kinase receptor belonging to the family of epidermalgrowth factor receptors (EGFRs).There are four members in the family:HER1 (EGFR), HER2, HER3 (ErbB-3) andHER4(ErbB-4).All have samemolecular structure with an extracellular ligand-binding domain, an intracellular domain with tyrosine kinase activity (excepting the HER3)and a short trans-membrane domain.Signal transduction for cell proliferation and processing is initiated if ligands bind to extracellular domain through homodimerization as well as heterodimerization. HER2 does not bind to any known ligand, but it is the preferred heterodimerization partner forother members of the HER family.

A gene located on long arm of chromosome 17 encodes HER2. Topoisomerase IIa genes are located adjacent to this gene and it is a oncogene. HER2 enact as an oncogene in gastric and other cancer by highlevel of amplification and thereby stimulates protein overexpression in the cell membrane and provide good microenvironment for growth of malignant cell.

Initially,HER2 overexpression/amplification was studied well in breast cancer and detected in invasive breast cancers in about 10%–34%.Presence of this overexpression was correlated with the clinical outcome, and found to be poor prognostic marker. Further studies,reported that this marker constitutea predictive factor of poor response to chemotherapy and endocrine therapy.

Later, HER2overexpression and/or amplificationhave also been observedin many cancers like colon, bladder, ovarian, endometrial, lung, uterine cervix,head and neck, esophageal and gastric carcinomas.

A monoclonal antibody, Trastuzumab (Herceptin) was then developed against HER2 whichby directly binding the extracellular domain of the receptor specifically targets HER2 protein. Clinical studied demonstrated that this monoclonal antibody increases the survival rates in both primary and metastatic HER2-positive breast cancer patients. This advantage of trastuzumab made the researcher to study the efficacy of it in otherHER2-positive cancers, including gastric adenocarcinomas.

For the past 2 decade, large number of studies had been published on HER2 positivity in gastric cancer and many studied the correlation between HER2-postivity and survival with/without association with important clinicopathological characteristics.

In 1986, Sakai K et al[40] first described the amplification of HER2 protein in gastric cancer using immunohistochemistry (IHC).Then, in 1990's case seriesstudied the incidence of positivity of HER2 in tumors using polyclonal antibodies directed against various domains of HER2 protein and reported the incidence rate being 9%–38%. From 2002 onwards, IHC using monoclonal antibody with/without gene amplification by FISH is being done.The commercially available kit is called HercepTest.

On systemic review of articles related to HER2,so far 48 articles comprising 17,338 patients were published. Out of these, 38 article comprising 11,860 patients studied the correlation between the HER2 status and relevant clinico-pathological variables such as vascular& tumorinvasion,lymph node involvement, metastases status, stage of diseaseand survival. The weighted mean HER2-positive status from the articles was 17.9% (95% CI; 14.8 to 20.9) ranging from 4.4% to 53.4%. Except two articles (also included OGj tumor), all articles studied cancer localized to stomach.

Study	**Country**	**N**	**HER2 positivity**	**Prognosis Information**
Yonemura Y et al(1991)[41]	Japan	260	11.9	++
MizutaniT et al(1993)[42]	Japan	226	14.0	++
Chariyalertsak et al(1994)[43]	Thailand	309	11.7	+
H Allgayer et al. (2000)[44]	Germany	189	53.4	++
JPintoet al(2002)[45]	Portugal	157	15.3	++
KE Lee et al. (2003)[46]	Korea	841	16.9	-
GZ Yu et al. (2009)[47]	China	1143	28.0	+
H Grabsch et al. (2010)[48]	UK	506	44	-
Kim et al. (2011)[49]	Korea	2009	13.8	++
Y Wang et al. (2011)[50]	China	436	20.6	++
Sekaran et al.(2012)[51]	India	52	44.2	NA

Correlation of HER2 expression with pathologic variables

Several studies in 1990s reported high correlation between HER2 expression and intestinal type of gastric cancer. More recent studies also confirms this as follows

Study N	**Histologic type**				**Localization**		
	Intestinal (%)	**Diffuse (%)**	**Mixed (%)**	**P value**	**GEJ (%)**	**Gastric (%)**	**P value**
Tanner et al[52] - 231	21.5	2	5	0.005	24	12	-
Gravalos et al[53]. 166	16	7	14	0.27	25	9.5	0.001
Lordick et al[54]. 1527	34	6	20	-	32	18	-

Detailed review on correlation of HER2 overexpressionwith clinical and pathological parameters were studied by various author over the past two decades. Most of the authors correlated the factors like age,genderdifference,location of tumor, histologicalpattern, clinicalstaging, lymph node metastasis, serosal involvement with and without HER2 positive gastric cancer.Results of these studies was summarized and given below(table-

).There was difference in results in these studies but generally more study reported that HER2 positivity correlates with intestinal type gastric cancer with advanced stage like nodal metastasis.Thus,it seems to be a prognosticate marker for advanced malignancy

List of Studies on clinicopathological correlation& their results.

Parameters		**Significant (positive correlation)**	**Non-significant (no correlation)**
Age	Young		Grabsch et al[48], Marx et al[55], Barros-Silva et al[56]
	Older	Grabsch et al[48],Marx et al[55], Barros-Silva et al[56]	
Gender	Male	Lee et al[65]	Wang et al[67],Marx et al[55], Barros-Silva et al.[56]
	Female		
Lauren classification	Intestinal	Grabsch et al[48],Marx et al[55], Barros-Silva et al[56].	Wang et al[67], Song et al[63],Pinto-de-Sousa et al[66].
	Diffuse/Mixed		
WHO classification	Papillary/tubular	Grabsch et al[48],Im et al[62],Lee et al[65].	Wang et al[67],Song et al[63],Pinto-de-Sousa et al[66].
Tumor grade	Well differentiated	Grabsch et al[48], Lee et al[65].	Wang et al[67],Marx et al[55], Song et al[63].
	Mod/poorly differentiated	Yu et al[59], Wang et al[67].	
Tumor location	Proximal	Yu et al[59], Pinto-de-Sousa et al[66].	Wang et al[67],Marx et al[55], Potrc et al[61],Garcia et al[64]Ghaderi et al[68],Allgayer et al[69]
	Distal		
Tumor	Early		Grabsch et al[48],Marx

depth (T)			et al[55],Yu et al[59].
	Advanced	Wang et al[67],Im et al[62].	
Lymph node metastases (N)	N0		Grabsch et al[48],Marx et al[55], Barros-Silva et al.[56], Yu et al[59].
	N1/N2/N3	Wang et al[67],Bazas et al[71],Potrc et al[61], Im et al[62].	
Presence of metastases (M)	Liver	Ougolkov et al[70]	
	Peritoneal	No study	
TNM staging	I/II		Marx et al[55], Barros-Silva et al[56].
	III/IV	Wang et al[67],Im et al[62], Ghaderiet al[68],Allgayer et al[69].	

Correlation between HER2 overexpression and survival

There were 39 studies which reported resection rates in both HER2 positive and HER2 negative gastric cancers.30 studies reported R0 resection.35 studies reported correlation of HER2 overexpression with overall survival.Out of 35,20 studies (57%) reported that there was no difference in overall survival in patients with and without HER2 amplification.13 studies (37%) demonstrated poorer overall survival that was statistically significant.Two studies reported longer survival but there was selection bias in those studies. From these studies, the median overall survival or 5-year survival rate were 21months and 33monthsrespectively in patients with and without HER2 overexpression, respectively

Number of studied that reported disease-free survival (DFS) was six. 3 studies reported significantly poorer DFS in patients with HER2 overexpression while the remaining otherthree studies reported no difference in DFS of patients with and without HER2 overexpression.Data were available on 3and 5- year DFS from four studies. The median 3-year DFS rate was 58% (range, 50–88%) and 86% (range, 62–97%) while median 5-year survival rate was 42% (range, 0–94%) and 52% (range, 5–87%) in patients with and without HER2 overexpressionrespectively.

Study Author	**3-Year disease free survival (%)**			**Median survival (Months)**		**5-Year survival (%)**		
	HER2 +ve	**HER2 -ve**	**p Value**	**HER2 +ve**	**HER2 -ve**	**HER2 +ve**	**HER2 -ve**	**P Value**
Grabsch[48]	NR	NR	NR	30	48	42	46	0.903
Barros[56]	NR	NR	NR	30	60	45	50	0.222
Im[62]	58	97	0.003	30	NR	35	77	0.004
Vizoso[72]	shorter DFS		0.01	HER2 +ve shorter OS				0.04
Allgayer[69]	50	80	0.002	19	35	NR	NR	0.016
Median	**58**	**86**		**21**	**33**	**42**	**52**	

Therapeutic Implication

Chemotherapy - Anti-HER2 therapy: trastuzumab
ToGA trial[73]

Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial had showed survival benefit.

The trial was conducted in 24 countries over 122 centersfor patients with gastro-oesophageal junction and gastric cancer if their tumors showed overexpression of HER2 protein by immunohistochemistry(IHC) or gene amplification by fluorescence in-situ hybridization(FISH). The primary endpoint was overall survival in all randomizedpatients who received study medication at least once.584 patients (n=294,n=290) were randomly divided into treatment(trastuzumab plus chemotherapy) and chemotherapy alone group and followed for 18.6 months.

The median overall survival was 13·8 months (95% CI 12–16) in treatment group vs 11.1 month in chemotherapy alone group(hazard ratio 0·74; 95% CI 0·60–0·91; p=0·0046) with no significant adverse side effect due to drug. Based this study,European and American cancer union has recommended trastuzumab as a new standard treatment option for patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer when combined with a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin.

Thus,HER2 positivity seems to be reliable prognosticate marker for gastric cancer and monoclonal antibody therapy against this receptor protein along with chemotherapy is a potential therapeutic option for gastric cancer of advanced stage disease with definite survival benefit.

The prevalence of this subtype of gastric cancer in India is not yet studied except in a study.So,we intended to study the frequency ofHER2 overexpression in our population and to find the correlation with clinical and pathological variables.

AIM OF THE STUDY

To study the incidence and clinico pathological correlation of HER2 positivity in gastric malignancy

Primary outcome

1. To find the incidence of gastric, oesophago gastric junction cancer with HER2overexpression.

Secondary outcome

1.Correlation of HER2 overexpression with clinical staging.

2.Correlation of HER2 overexpression with pathological variables.

MATERIALS AND METHODS

This is a hospital based prospective cohort study, done at Department Of Digestive Health and Sciences, Government Peripheral hospital, Anna nagar, Chennai from June 2013 to February 2014.

All consecutive patients of gastric adenocarcinoma diagnosed during the study period were enrolled in the study. The diagnosis of gastric adenocarcinoma was made in endoscopic gastric biopsy specimen by the pathologist.Written informed consent was obtained from all participating subjects in regional language and privacy of illness wasensured.

All enrolled patients were assigned a case number and details like age, residential address along with contact number were noted. Clinical presentation symptoms with duration and relevant details of risk factors for gastric cancer like smoking, alcohol intake ,previous peptic ulcer disease, dietary history, socioeconomic status and previous gastric surgery details were noted. All positive signs on clinical examination and relevant blood investigations were noted.

Tumors were tested for HER2 status using immunohistochemistry (IHC) on formalin-fixed paraffin-embedded sectionsof biopsy specimen.Antibody against HER2 ,Hercep test kit (polyclonal rabbit anti tumor cer-B2 onco-protein-DAKO) was used and was done in medall diagnostic lab,Chennai.

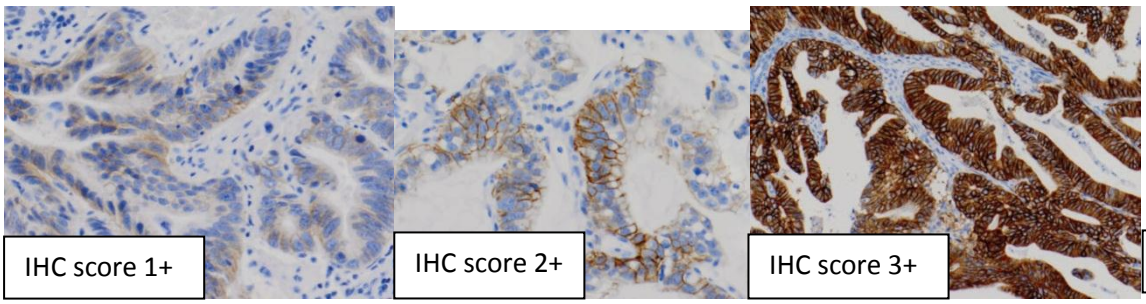
Scoring for HER2 overexpression was done by two independent experienced pathologist based on validated Hofmann et al. scoring for gastric adenocarcinoma as given below.

Expression of HER2 was graded from score 0 to 3 and score of 3+ was considered as positive for HER2 overexpression.

.

Pattern	Score/Classification
No reactivity or membranous reactivity in <10% of cells	0/negative
Faint/barely perceptible membranous reactivity in >10% of cells; cells are reactive only in part of their membrane	1+/negative
Weak to moderate complete or basolateral membranous reativity in >10% of cells	2+/equivocal
Moderate to strong complete or basolateral membranous reativity in >10% of cells	3+/positive
Biopsy samples with cohesive IHC3+ or FISH+ clones, irrespective of size (even if <10%)	3+/positive

Consensus panel recommendation for HER2 evaluation og gastric cancer from Hoffman validation study[74]



Overexpression of HER2 in gastric adenocarcinoma was evaluated and correlated with various clinical and pathological parameters like age,gender,and histological parameters like grading of differentiation,type of adenocarcinoma(intestinal vs Diffuse).To stage the disease, CT scan of abdomen with lower thorax and/or ascitic fluid analysis for malignant cells

were done. Clinical staging was done as per TNM classification as follows where T = Tumor depth, N= Nodal involvement and M= metastasis

	N0	N1	N2	N3	M1(Any N)
Tis	Stage 0	-	-	-	-
T1	Stage IA	IB	II	IV	IV
T2	IB	II	IIIA	IV	IV
T3	II	IIIA	IIIB	IV	IV
T4	IIIA	IV	IV	IV	IV

T1= tumor confined to the mucosa or submucosa,
T2 = tumor involves of the muscularispropria,
T3 = tumor invades through the serosa, and
T4 = tumor invasion into adjacent organs or structures.
N0 = no lymph node involvement,
N1 = involvement of 1 to 6 regional lymph nodes,
N2 = involvement of 7 to 15 regional lymph nodes,
N3= involvement of more than 15 regional lymph nodes

M1=Presence of distant metastasis

Follow-up

Study population wasfollowed up during the study period. Treatment received by the patient like curative or palliative surgery with or without chemotherapy or palliative supportive care was recorded. Patient was asked to follow up every 3 months. If not followed up after 4 months, patientwas contacted through telephone and enquired about treatment and survival details.

RESULTS

A total of 70 patients with histopathological diagnosis of gastric adenocarcinoma were enrolled during the study period. 9 patients were not willing for HER2 immunohistochemistry testing.61 patients in whom test was done were included for study analyses.

Statistical analysis was done using SPSS software (Version 17).Univariate and Multivariate analysis was done. Chi square value and fischer exact probability were used to compare variables of two study arms. P value <0.05 is considered statistically significant

AGE DISTRIBUTION

Majority of patients were in age group >60 years and middle age. 5 patients were < 40yrs of age with youngest patient being a 19 years old boy.

Age	**Number of subjects**	**Percentage**
<40 yrs	5	8.3
40-60 yrs	24	39.3
>60 yrs	32	52.4

Table.1 : Age group distribution of study population.

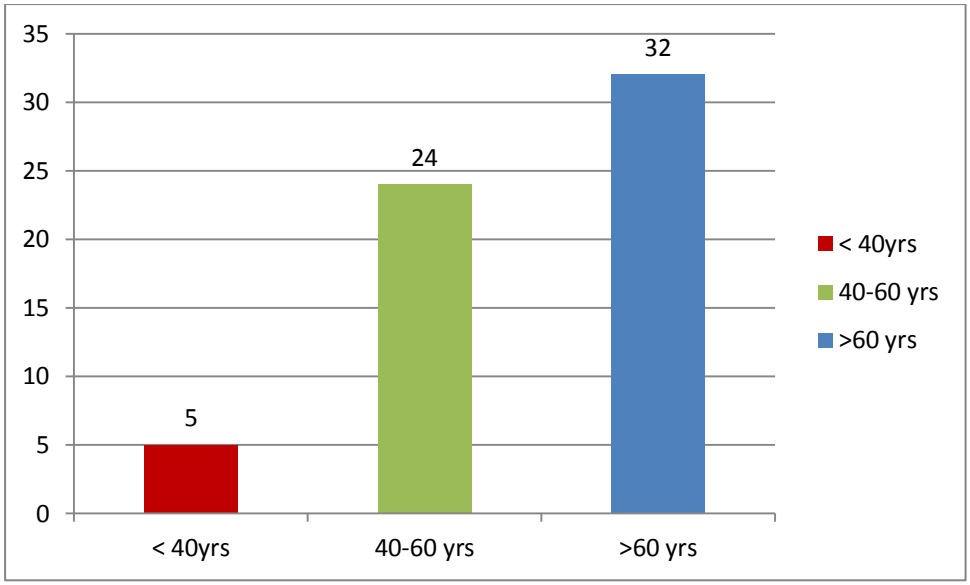


Fig.1: Age group distribution

GENDER DISTRIBUTION

Male predominates with 53 out of total of 61 patients with sex ratio of 6.7 : 1.

Gender	Number of subjects	Percentage
Male	53	87
Female	8	13

Table.2 : Gender distribution of study population

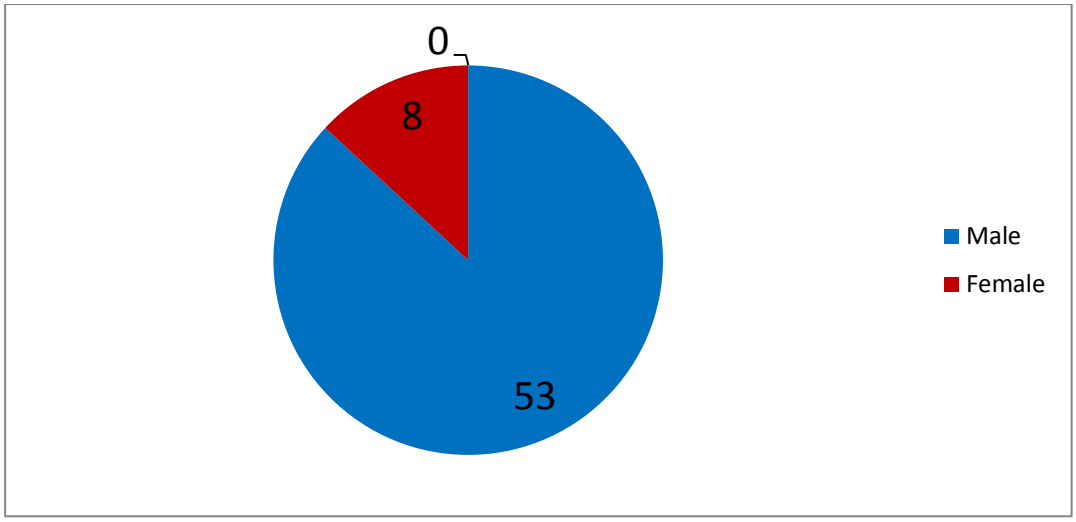


Fig.2 : Gender distribution.

CLINICAL PRESENTATION

Constitutional symptom like loss of weight and appetite was the commonest presenting complaint accounting for 80-90% followed by abdominal pain in 77% and vomiting in 64%.[Table.3 & Fig.3]

Presenting Complaints	**Number of subjects**	**Percentage**
Abdominal Pain	47	77
Vomiting	39	64
Early satiation	26	43
Loss of weight	56	92
Loss of appetite	49	80

Table.3:Frequency of presenting complaints.

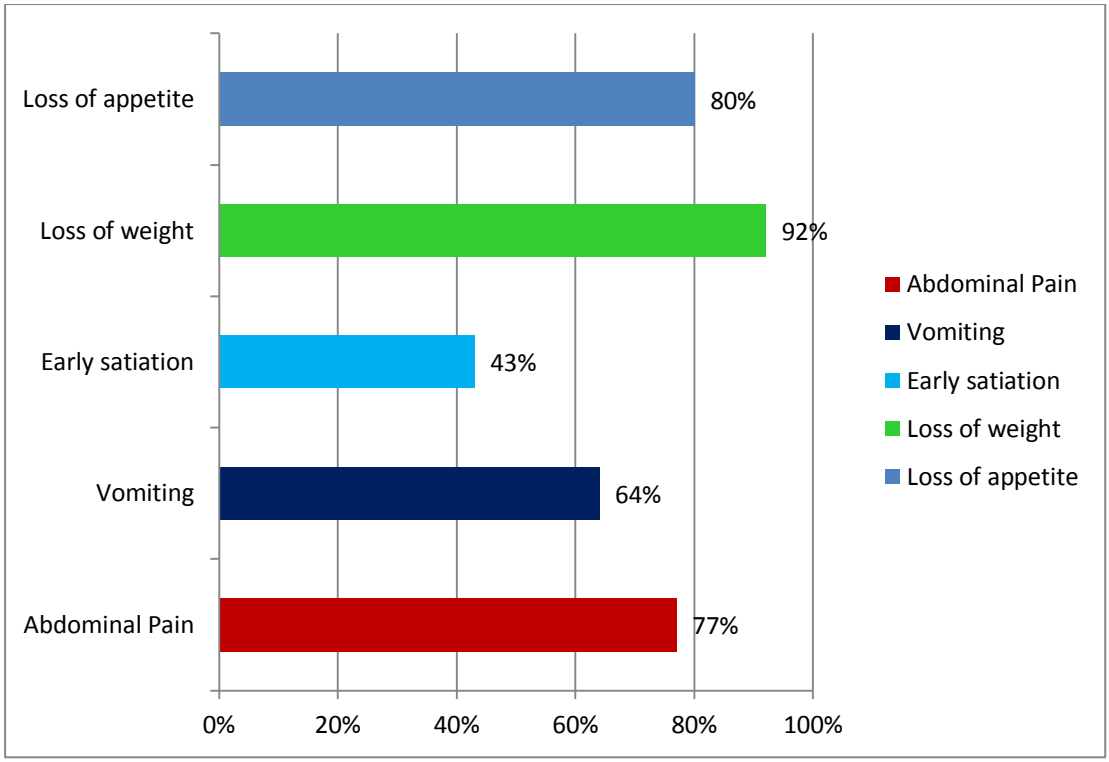


Fig.3: Presenting complaints and its frequency.

RISK FACTORS

In our study major risk factor identified were smoking and chronic alcohol intake with association of about 72% and 66% respectively. Previous history of peptic ulcer was present in about one fifth of subjects. Past history of gastric surgery was present in only 4 out of 61 patients.

Risk factors	**Number of subjects**	**Percentage**
Smoking	44	72

Alcohol	40	66
Previous Peptic ulcer disease	13	21.3
Previous Gastric surgery	4	6.5

Table.**4**: Risk factors present in study population.

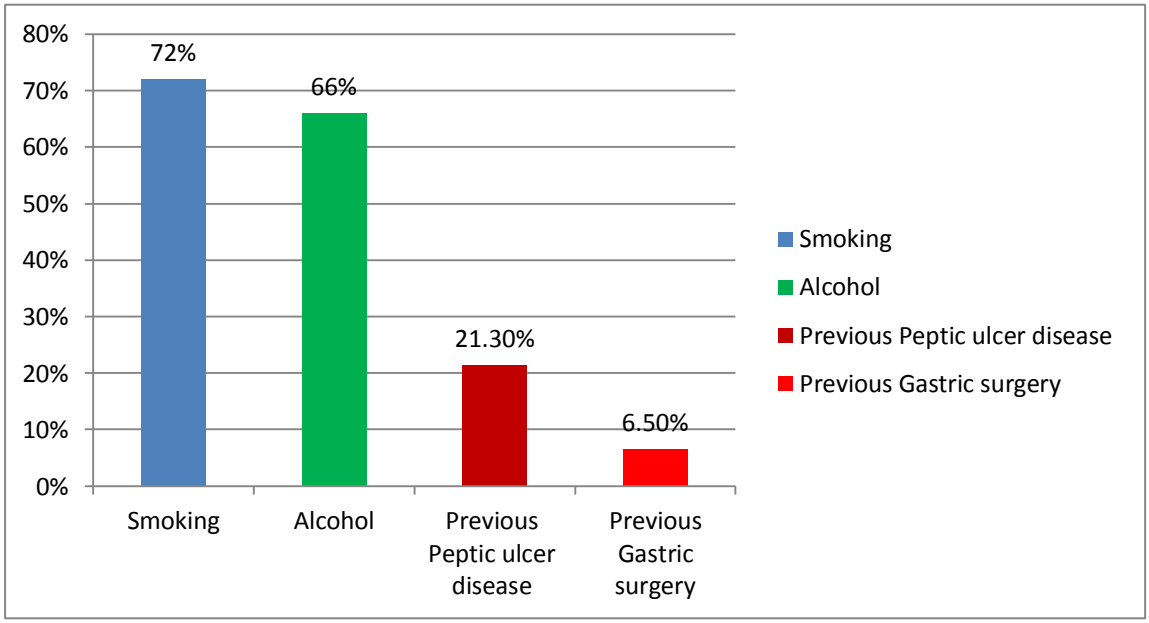


Fig.4: Depicts risk factors and its frequency.

CLINICAL SIGNS

Clinical signs were less frequent compared to symptoms at time of presentation. Mass palpable per abdomen was elicit able in 13% and sign of

distant metastases like hepatomegaly due to metastasis and ascites was present in 21% and 15% respectively.

Clinical Signs	Number of subjects	Percentage
Mass per abdomen	8	13.1
Hepatomegaly	13	21.3
Ascites	9	14.8

Table.5: Major clinical sign and its frequency.

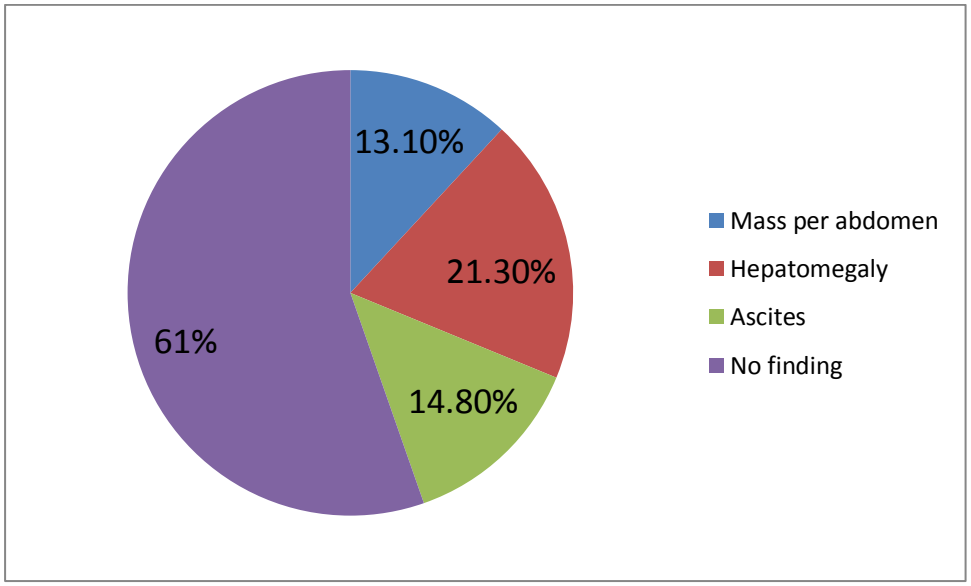


Fig.5: Frequency of clinical signs present in study population.

LOCATION OF TUMOR

Only one patient presented with growth at gastric cardia and rest 60 patients presented with tumor in distal stomach. Antrum was the commonest

location of tumor(about 40 percent), followed by body and involvement of both body and antrum in 26 and 23 percent respectively.

Diffuse infiltrative type of gastric cancer (linitusplastica) was noted in 6 patients accounting for about 10 percent.

Site	**Number of subjects**	**Percentage**
Proximal	**1**	**1.6**
- Osophagogastric Junction	1	
- Gastric Cardia	0	
Distal	**60**	**98.4**
-Body	16	26.3
-Antrum	24	39.3
-Both Body and antrum	14	23.0
- Diffuse Linitusplastica	6	9.8

Table.6: Site of location of tumor and its frequency.

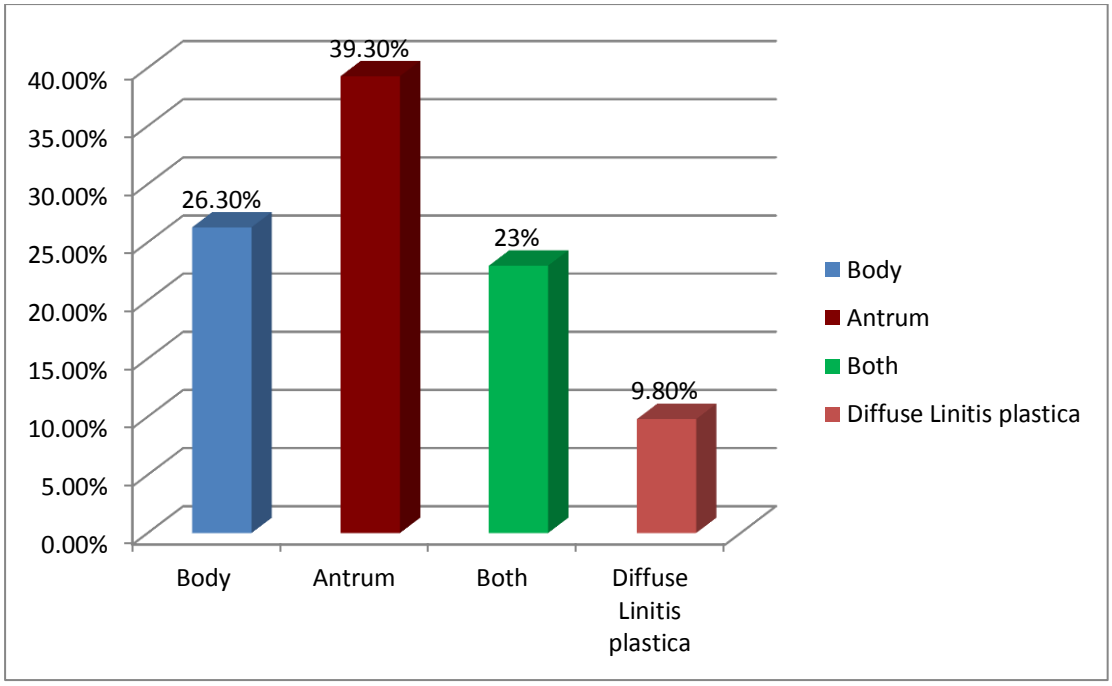


Fig.6: Percentage distribution of gastric cancer based on location.

GRADING OF TUMOR

Histologically tumor cells were graded depending on degree of differentiation as well differentiated to poorly/ undifferentiated cells. In our study, 46% of patients had moderately differentiated tumor cells and 21% had poorly differentiated tumor cells. One patient had signet ring type adenocarcinoma and two patients had scirrhous type adenocarcinoma.

HPE	Number of subjects	Percentage
Well Differentiated	13	21.2

Moderately Differentiated	28	46.0
Poorly Differentiated	17	27.8
Diffuse type-poor cohesion	3	5.0

Table.7: Different grades of differentiation on histology and its prevalence.

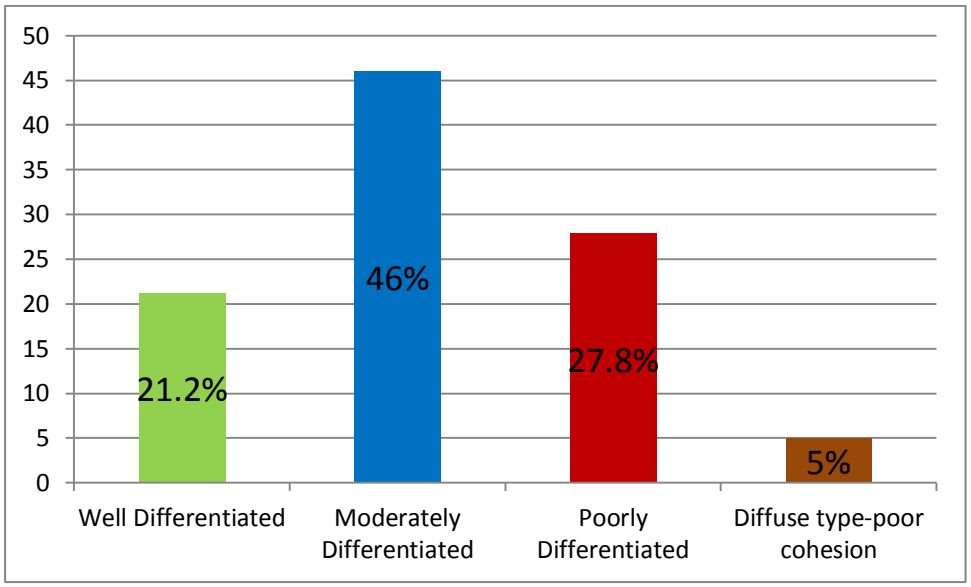


Fig.7: Percentage distribution of grades of differentiation.

CLINICAL STAGING

Based on oral and intravenous Contrast enhanced CT abdomen, clinical staging was made. None was presented at early stage of disease. Majority presented at advanced stage of disease with 46% in stage III and 28% in stage IV.

Clinical stage	Number of subjects	Percentage
I	0	0
II	16	26.2
III	28	45.9
IV	17	27.9

Fig.8: Frequency of clinical stages of gastric cancer in study population.

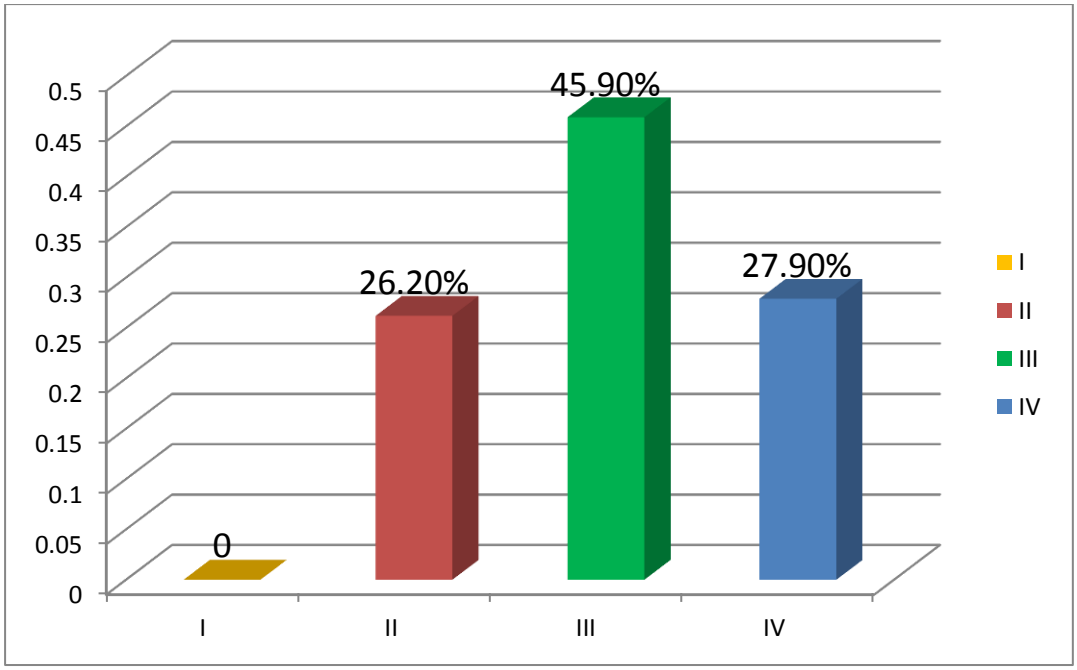
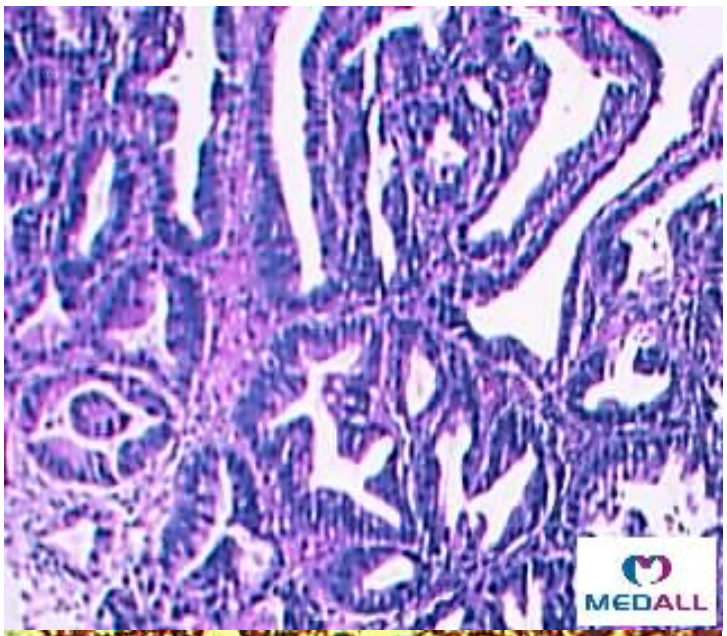


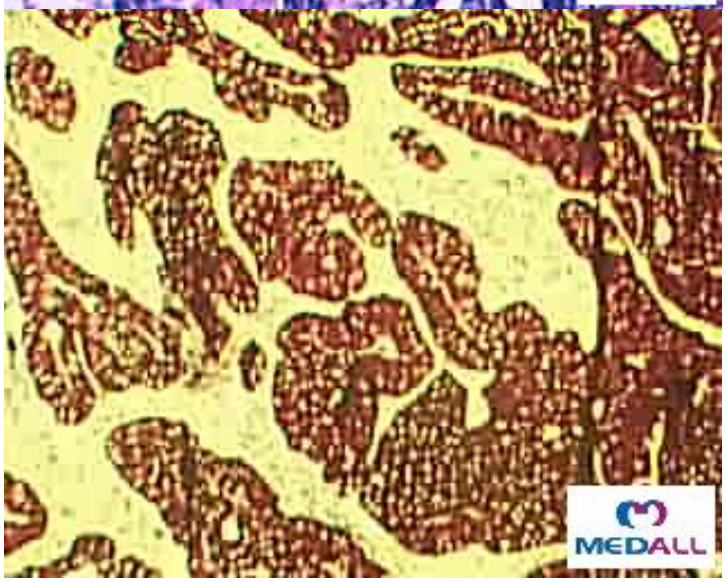
Fig.8: Percentage distribution of clinical stages

HER2 OVEREXPRESSION STATUS.

Out of 61 cases, HER2 overexpression was positive with 3+ staining in 21 patients, constituting 34.4% of study population. One example shown below.



Case no 43 : HPE showing moderately differentiated adenocarcinoma



Case no 43 : Immunohistochemistry of same patient showing 3+ staining pattern

HER2 Overexpression	Number of patients	Percentage

Positive(3+)	21	34.4
Negative(less than 3+)	40	65.6

Fig.9: Frequency of HER2 overexpression in study population.

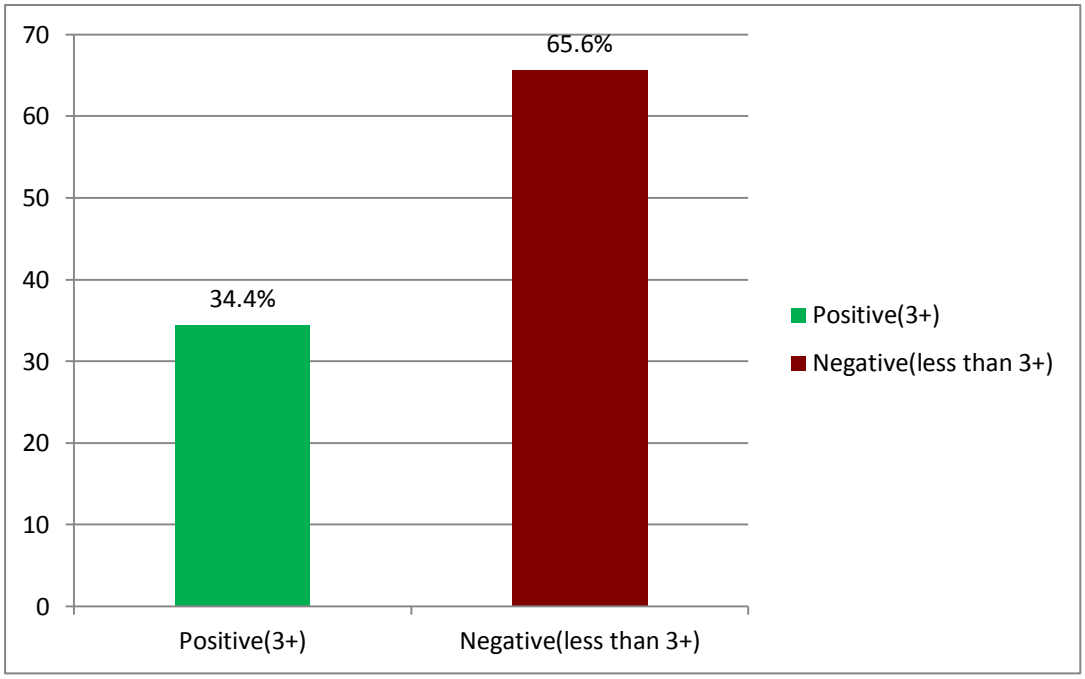


Fig.9: Percentage of HER2 positivity in study population.

CORRELATION OF HER2 OVER EXPRESSION WITH VARIOUS CLINICAL AND PATHOLOGICAL VARIABLES.

CORRELATION WITH AGE OF PRESENTATION AND GENDER

By univariate analysis HER2 overexpression was correlated with age and gender. Results (Table.10 & 11) revealed that there was no correlation between these variables and HER2 positivity.

Table.10:Univariate analysis of HER2 overexpression in various age groups.

Age	**HER2 overexpression**		**Chi square value**	**P value**
	Yes	No		
<40	4	1		
40-60	8	16	5.18	0.07
>60	9	23		

Table.11: Univariate analysis of HER2 overexpression in relation to gender.

Gender	**HER2 overexpression**		**Odds ratio (95% CI)**	**Fischer exact probability**	**P value**
	Yes	No			
Male	17	36	0.47 (0.08-2.62)	0.99	0.27
Female	4	4			

CORRELATION WITH LOCATION OF TUMOR.

There was no correlation between HER2 overexpression and location of tumor as depicted in below table (P value =0.656)

Table.12: Correlation of HER2 overexpression with location of tumor.

Site	HER2 overexpression		Odds ratio (95% CI)	Fischer exact probability	P value
	Yes	No			
Proximal	0	1	0.00 (0.00-34.34)	0.53	0.656
Distal	21	39			

CORRELATION WITH CLINICAL STAGING.

As stage of disease increases, the positivity of HER2 overexpression increases significantly. There was statistical significance (p value = 0.0003) correlation with clinical staging.

Table.13: Frequency distribution showing HER2 overexpression in relation to gastric cancer staging

Stage	Number of cases (%)	HER2 overexpression (n[%])		Chi square	P - value
		Yes	No		
I	0	-	-	-	-
II	16 (26.2)	1 (6.3)	15 (93.7)	15.9	0.0003
III	28 (45.9)	8 (28.6)	20 (71.4)		
IV	17 (27.9)	12 (70.6)	5 (29.4)		

CORRELATION WITH GRADING OF TUMOR.

HER2 overexpression was more prevalent in intestinal type of cancer and in particular moderately to poorly differentiated type compared to well differentiatedtype.As degree of differentiation increase, HER2 positivity increases with statistical significance.

Table.14: Correlation of HER2 overexpression with grades of differentiation of tumor cells.

HPE	HER2 overexpression	Chi square	P value

	Yes	No	**value**	
Well Diffentiated	1	12		
Moderately differentiated	8	20	10.40	0.015
Poorly differentiated	10	7		
Undifferentiated/ Poorly cohesive type	2	1		

FOLLOW UP RESULTS

During the one year study period, only seven patients had curative resection for cancer and none were in HER2 positive group. About 50% of HER2 positive patients were in advanced stage where palliative surgery and chemotherapy was not feasible due to poor performance status of patient. Those who received palliative chemotherapy were not able to complete the full course due to other complications and death. Only 2 patients in HER2 positive study arm were surviving till this study period, one patient was at 6^{th} cycle of chemotherapy and other patient was just started the first cycle of chemotherapy. In comparison other study arm, the survival rate was less in HER2 positive group (9.5% vs 15%)

Treatment received	**HER2 overexpression**	
	Yes **N=21**	**NO** **N=40**

Underwent curative surgery and chemotherapy	0	7
Palliative surgery and received full course palliative chemotherapy	5	11
Palliative surgery and received partial palliative chemotherapy	6	17
Palliative Supportive care	10	5
Survival at end of study period (%)	2(9.5%)	6(15%)

DISCUSSION

Gastric cancer is a potentially curable disease if diagnosed at early stage, where patient are mostly asymptomatic. And when patient with gastric cancer have clinical symptoms and present to clinician, majority are already in advanced stage of disease where curative treatment is not possible. Screening for detection of early cancer is proved to be not a cost effective strategy except in high prevalence area. Thus, in majority when diagnosis of gastric cancer is made, patient is left with the only treatment option of palliative surgery with chemotherapy. In addition, the response to chemotherapy is not uniform in all patients even at same stage of disease due to biological aberrations in tumor cells and this leads to poor survival results. Researches have shown that specific subgroup of patient with HER2 overexpression were found to have survival benefit if they were treated with antibody against HER2 receptor. With this data, National comprehensive cancer network (NCCN) recommends HER2 testing as part of routine work up in gastric cancer patients. In India, the study on prevalence of subgroup of gastric cancer with HER2 overexpression is very scanty with only one study by Sekaran et al.

Since our study is a hospital based study, the true incidence of gastric cancer in population cannot be ascertained but in our study we observed that there is increasing incidence of cancer in younger age group (<40 yrs) that

constitutes about 8% of total case. Anderson et al also reported that there is unexplained increase in incidence in younger individuals.[74] We had more male predominance of 6.7 : 1 as compared to GLOBOCAN 2008 data of 2 : 1 may be due to referral bias.

Frequency of presenting symptoms like loss of weight and abdominal pain was about 90% and 70% in our study compared to 60% and 50% respectively in Waneboet al.[75]This may be explained by patient's habit of seeking medical advice at delayed phase. Smoking habit was present in 72% of our subjects as reported in various studies from European and Asia.[76-78] We observed less prevalence of other risk factors like previous peptic ulcer disease and previous gastric surgery in our study cohort.

36.1% of our study population present with metastasis at time of diagnosis. Liver was the most common site for metastasis followed by peritoneal in 21% and 15% respectively comparable to the previous studies.[79-81] In our study intestinal type of gastric cancer was the predominant type as comparable to various population based studies.[45-49] We had more patients (74%) in advanced stages (III & IV) of disease, may be due to late referral.

HER2 Overexpression and correlation.

In our study cohort of 61 patients, 21 patients had overexpression of HER2 accounting for 34.4%.This was comparable to various studies worldwide and from systemic review which reported the incidence in range of 9 – 38%.[41-49]Sekaran et al ,the other only study from India reported the incidence being 44.2%(23 out of 52 cases).No significant correlation was demonstrable between HER2 positivity and age and gender distribution of cancer and this was comparable to the studies done by Lee et al[46] and Wang et al.[50]

We had almost all ,except one patient withtumor located in distal stomach either antrum or body, hence could not be able to correlate the HER2 overexpression with location of tumor. Previous studies had reported that HER2 amplification was slightly more in proximal tumor compared to distal tumor.[52-53] but, this finding was not consistently seen in other studies.[54]

In our study, we had observed that there was definite correlation of HER2 overexpression with clinical stage of disease. As stage of disease advances, the frequency of Her2 overexpression increases with statistical significance(p value =0.003). This was comparable to the studies done by Marx et al, [55] Barros-Silva et al, [56] and Park et al[82] which all reported that there wasno significant correlation of HER2 overexpression with stage I/II disease. Study by Wang et al.[67], Zhang et al.[58]Ghaderi et al., [68]Allgayer et al [69] reported that there is significant association between HER2 overexpression and stage III/IV disease.

Our study demonstrated that HER2 overexpression was more in intestinal type of gastric cancer than diffuse type with frequency of 43.5%, 47% and 9.5% in intestinal,mixed,diffuse type respectively. A Korean study[82] also reported this correlation. ToGA trial[73] also had similar results with frequency of HER2 overexpression as follows- intestinal 34 %, diffuse 6 %, mixed 20 %.Sekaran et al reported equal distribution of HER2 positivity in both intestinal and diffuse type of gastric cancer.

Follow up results of our study cohort cannot be extrapolated because mean duration offollow up was only 3.1 months. With the available data, majority of patients(n= 21) in HER2 positivity group(50%) had advanced stage of disease where palliative supportive care was the only treatment option left. One patient in this group had completed palliative chemotherapy and on follow up. One patient had just started first course of chemotherapy. All other patients in HER2 positivity arm, succumbed to death with incomplete course of chemotherapy or medically unfit for chemotherapy. On HER2 negativity arm(n= 40), we have 6 patients on follow up, out of which one had curative resection and completed chemotherapy,4 patients were receiving chemotherapy and one patient was in supportive care.Thus,HER2 positivity was associated with poor survival outcome.

Limitation of the Study.

Number of study population is less to extrapolate the results. Study warrants need for long duration follow up of study population. The associated comorbid medical conditions that can affect morbidity and mortality were not attested. Newer chemotherapeutic agents and good supportive care are not made available to the patients due to non-availability and financial constraints.

CONCLUSION

1. This study is first of its kind done in our part of country.

2. Distal gastric cancer is more common than proximal gastric cancer.

3. Majority of patients(74%) present at advanced stage of disease.

4. In our study, HER2 positivity is observed in 34.4%.

5. HER2 overexpression is positively correlated with advanced stage of gastric cancer with statistical significance.

6. HER2 positive gastric cancer patients seem to have poor survival outcome.

7. In future, HER2 testing may be a part of routine work up in gastric cancer patient to prognosticate the survival outcome.